

***Septobasidium aquilariae* sp. nov. from Yunnan, China**

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ABSTRACT—A new fungal species, *Septobasidium aquilariae*, is proposed based on a combination of morphological features and molecular evidence. The species is characterized by an annual growth habit; a resupinate coriaceous basidiocarp with a cream to pale brown surface; a monomitic hyphal system with thick-walled generative hyphae bearing simple septa; reniform, hyaline, thin-walled, smooth basidiospores measuring $11\text{--}19 \times 4\text{--}7.5\ \mu\text{m}$; and haustoria consisting of irregularly coiled hyphae. The fungus was found associated with *Pseudaulacaspis* sp. on *Aquilaria sinensis*. Sequences of internal transcribed spacer region (ITS) were analysed maximum likelihood, maximum parsimony, and Bayesian inference methods. The phylogenies strongly supported *S. aquilariae* in a monophyletic lineage (ML = 100%; MP = 100%; PP = 1) and grouped with “*S. cokeri*”.

KEY WORDS—felt fungus, *Pucciniomycetes*, *Septobasidiaceae*, *Septobasidiales*, taxonomy

Introduction

Septobasidium Pat. (*Septobasidiaceae*, *Septobasidiales*), erected by Patouillard (1892), is a large, cosmopolitan genus characterized by resupinate basidiocarps with a white to cream, yellowish brown or brown hymenophore, a monomitic hyphal system with simple septa, with or without probasidia, 2–4 celled cylindrical, curved, or straight basidia, basidiospores that are hyaline, thin-walled, smooth, and cylindrical or fusiform, and haustoria consisting

of coiled or spindle-shaped hyphae (Patouillard 1892, Couch 1929). About 300 species have been accepted in the genus worldwide (Patouillard 1892; Bresadola & Saccardo 1897; Burt 1916; Lloyd 1919; Couch 1929, 1935, 1938, 1946; Yamamoto 1956; Gómez & Henk 2004; Henk 2005; Lu & Guo 2009a,b, 2010a,b, 2011; Lu & al. 2010; Chen & Guo 2011, 2012; Li & Guo 2013, 2014).

Molecular systematics have played a powerful role in inferring phylogenies within fungal groups since the early 1990s (White & al. 1990, Binder & al. 2013, Dai & al. 2015, Choi & Kim 2017). However, molecular studies involving *Septobasidium* are rare (Henk & Vilgalys 2007, Zhao & al. 2017). One phylogenetic study of a single origin of insect symbiosis in the *Pucciniomycetes* suggested that there is little or no support for *Septobasidium* as a monophyletic group (Henk & Vilgalys 2007). Zhao & al. (2017) introduced a six-gene phylogenetic overview of *Basidiomycota* and allied phyla and confirmed that *S. carestianum* Bres. nested within the *Septobasidiales* and grouped with *Helicobasidium mompa* Nobuj. Tanaka and *Thanatophytum crocorum* (Pers.) Nees.

During disease investigations on *Aquilaria sinensis* in southern China, a new *Septobasidium* taxon was found that could not be assigned to any described species. In this study, we expand samplings from previous studies to examine the taxonomy and ITS phylogeny of this new species within the *Septobasidium*.

Materials & methods

The specimens studied are deposited at the herbarium of Southwest Forestry University, Kunming, China (SWFC). Macromorphological descriptions are based on field notes. Colour terms follow Petersen (1996). Micromorphological data were obtained from dried specimens observed under a light microscope. Measurements are presented as: L = mean spore length, W = mean spore width, Q = range of L/W ratios, n = number of spores/number of specimens.

Genomic DNA was obtained from dried specimens using the EZNA HP Fungal DNA Kit, according to the manufacturer's instructions with some modifications. A small piece of dried fungal specimen (about 30 mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 ml centrifuge tube, suspended in 0.4 ml of lysis buffer, and incubated in a 65 °C water bath for 60 min. After that, 0.4 ml phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 ml supernatant was transferred to a new tube and mixed with 0.45 ml binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 ml inhibitor removal fluid was added in AC for a centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 ml washing buffer, the AC was transferred to a clean centrifuge tube,

TABLE 1. Species, specimens, and ITS sequences used in *Septobasidium* molecular analyses.

SPECIES	SAMPLE	GENBANK ITS	REFERENCE
<i>Helicobasidium mompa</i>	DAH h1	DQ241472	Henk & Vilgalys 2007
<i>Pachnocybe ferruginea</i>	DAH pf1	DQ241473	Henk & Vilgalys 2007
<i>Septobasidium alni</i>	DAH FP3	DQ241441	Henk & Vilgalys 2007
<i>S. aquilariae</i>	CLZhao 6610	MK802139	Present study
	CLZhao 6611 [T]	MK802140	Present study
	CLZhao 6612	MK802141	Present study
	CLZhao 6613	MK802142	Present study
	CLZhao 6614	MK802143	Present study
<i>S. arachnoideum</i>	DAH 025	DQ241443	Henk & Vilgalys 2007
<i>S. bogoriense</i>	998434	HM209414	Unpublished
<i>S. broussonetiae</i>	998436	HM209416	Unpublished
<i>S. burtii</i>	DAH 062	DQ241444	Henk & Vilgalys 2007
<i>S. carestianum</i>	DJM 644	DQ241448	Henk & Vilgalys 2007
<i>S. castaneum</i>	DAH 052	DQ241447	Henk & Vilgalys 2007
<i>S. cavarae</i>	DJM FP1	DQ241445	Henk & Vilgalys 2007
" <i>S. cokeri</i> "	DAH 061	DQ241449	Henk & Vilgalys 2007
<i>S. fumigatum</i>	DAH 005	DQ241451	Henk & Vilgalys 2007
<i>S. gomezii</i>	DAH 031	DQ241462	Henk & Vilgalys 2007
<i>S. grandisporium</i>	DAH 065	DQ241453	Henk & Vilgalys 2007
<i>S. hainanense</i>	998437	HM209417	Unpublished
<i>S. kameii</i>	998432	HM209412	Unpublished
<i>S. maesae</i>	998433	HM209413	Unpublished
<i>S. marianiae</i>	DAH 283b	DQ241456	Henk & Vilgalys 2007
<i>S. michelianum</i>	DAH FP5	DQ241457	Henk & Vilgalys 2007
<i>S. pallidum</i>	998435	HM209415	Unpublished
<i>S. pilosum</i>	DAH 020	DQ241458	Henk & Vilgalys 2007
<i>S. pinicola</i>	DAH 013	DQ241459	Henk & Vilgalys 2007
<i>S. pseudopedicellatum</i>	DAH 044	DQ241460	Henk & Vilgalys 2007
<i>S. ramorum</i>	DAH 045a	DQ241450	Henk & Vilgalys 2007
<i>S. septobasidioides</i>	DAH 032	DQ241461	Henk & Vilgalys 2007
<i>S. taxodii</i>	DAH 194C	DQ241466	Henk & Vilgalys 2007
<i>S. velutinum</i>	DAH 024	DQ241467	Henk & Vilgalys 2007
<i>S. westonii</i>	DAH FP2001	DQ241468	Henk & Vilgalys 2007
<i>S. wilsonianum</i>	DAH 037	DQ241469	Henk & Vilgalys 2007

and 100 ml elution buffer was added to the middle of adsorbed film to elute the genomic DNA. ITS region was amplified with primer pairs ITS5 and ITS4 (White & al. 1990). The PCR procedure was as follows: initial denaturation at 95 °C for 3 min; followed by 35 cycles of 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min; and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (TABLE 1).

Sequencher 4.6 was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Kato & Toh 2008; <http://mafft.cbrc.jp/alignment/server/>) using the “G-INS-I” strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 23722). Sequences of *Helicobasidium mompa* and *Pachnocybe ferruginea* Berk. obtained from GenBank were used as outgroups to root trees following Henk & Vilgalys (2007) in the ITS analysis (FIG. 1).

Maximum parsimony analysis was applied to the ITS dataset sequences. Approaches to phylogenetic analysis followed Zhao & al. (2013) and Song & al. (2016), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR+G branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BP) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAXML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org; Miller & al. 2009). Branch support (BS) for ML analysis with GTR+G+I model of site substitution including estimation of Gamma-distributed rate heterogeneity and a proportion of invariant sites. The branch support was evaluated with bootstrapping method of 1000 replicates.

MrModeltest 2.3 (Posada & Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR+G+I) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 3 million generations (FIG. 1) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap (ML)>70%, maximum parsimony bootstrap (MP) >50%, or Bayesian posterior probabilities (PP) >0.95.

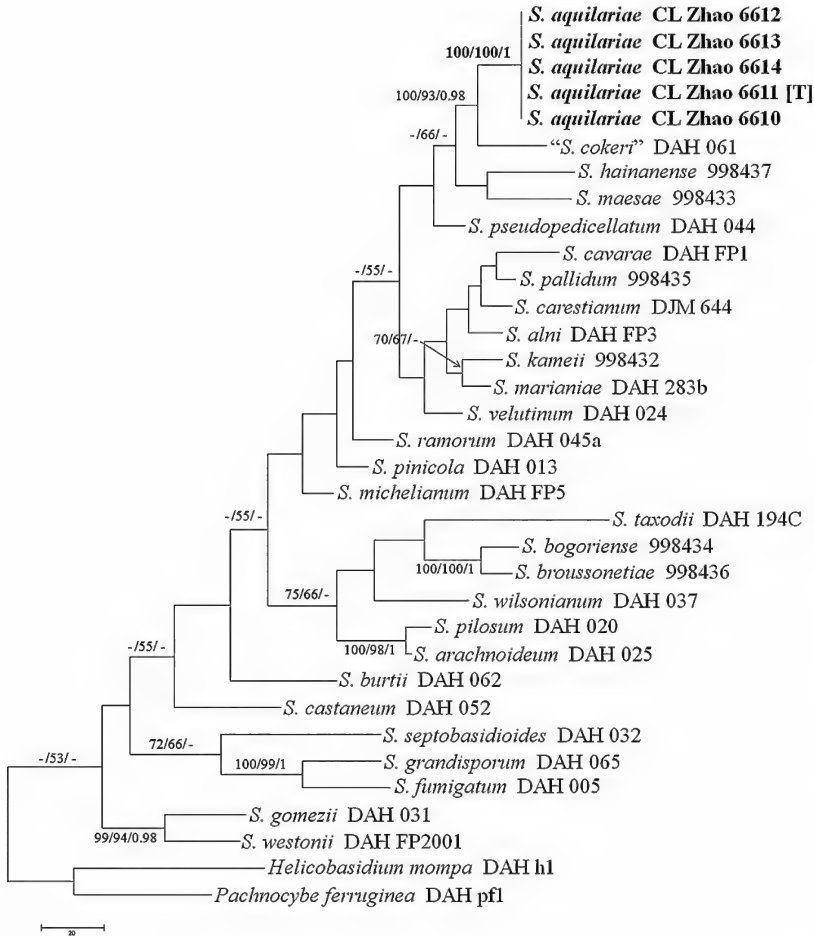


FIG. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Septobasidium aquilariae* and related species in *Septobasidium* based on ITS sequences, with *Helicobasidium mompa* and *Pachnocybe ferruginea* as outgroup. Branches are labeled with maximum likelihood bootstrap >70%, maximum parsimony bootstrap >50% and Bayesian posterior probabilities >0.95.

Phylogeny

The ITS dataset included sequences from 34 fungal specimens representing 29 species. The dataset had an aligned length of 617 characters, of which 315 are constant, 106 are variable and parsimony-uninformative, and 196 are parsimony-informative. Maximum parsimony analysis yielded 12

equally parsimonious trees (TL = 1143, CI = 0.434, HI = 0.566, RI = 0.454, RC = 0.197). Best model for the ITS dataset estimated and applied in the Bayesian analysis: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.008724 (BI).

The Maximum Parsimony strict consensus tree (FIG. 1) inferred from ITS sequences within *Septobasidium* demonstrated that the new species formed a monophyletic lineage with strong support (ML = 100%; MP = 100%; PP = 1) and formed a sister clade with “*S. cokeri*” Couch [nom. inval.] and forming a group with *S. hainanense* C.X. Lu & L. Guo and *S. maesae* C.X. Lu & L. Guo.

Taxonomy

Septobasidium aquilariae C.L. Zhao, **sp. nov.**

FIGS 2, 3

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Differs from “*Septobasidium cokeri*” by its slightly brown to brown hymenial surface upon drying and its growth on *Aquilaria sinensis*.

TYPE: China. Yunnan Province: Xishuangbanna, Jinghong, Menghai County, in association with *Pseudaulacaspis* sp. on *Aquilaria sinensis*, 31 May 2018, CLZhao 6611 (Holotype, SWFC 006611; MK802140).

ETYMOLOGY: The specific epithet *aquilariae* (Lat.) refers to the plant genus on which all of the specimens were collected.

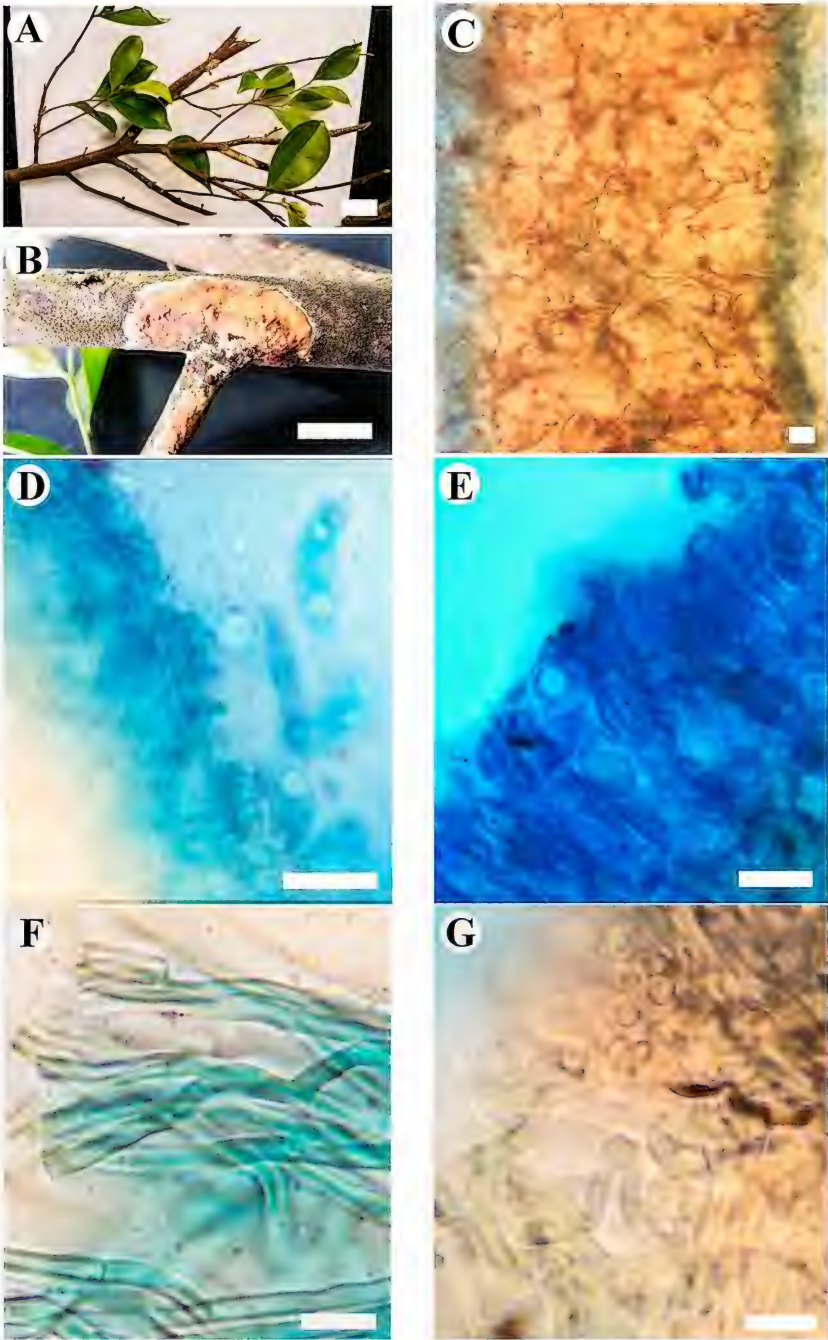
BASIDIOMATA on branches, annual, resupinate, hard to separate from substrate, becoming coriaceous upon drying, up to 8 cm long, up to 4 cm wide, up to 1 mm thick. Hymenial surface smooth, cream to pale brown when fresh, slightly brown to brown upon drying. Sterile margin indeterminate, white to cream.

HYPHAL STRUCTURE monomitic; generative hyphae with simple septa, pale brown, thick-walled. In section 800–1000 µm thick; subiculum pale brown, 50–150 µm thick; pillars brown, 300–400 µm high, 50–100 µm wide; hyphal layer pale brown, 150–350 µm thick; hymenium hyaline or brown, 70–100 µm thick.

BASIDIA arising directly from the hyphae without a probasidial cell, cylindrical, curved, or straight, hyaline, 2–4-celled, 15–26.5 × 4–6 µm.

BASIDIOSPORES reniform, hyaline, thin-walled, smooth, (10–)11–19(–20.5) × (3.5–)4–7.5(–8) µm, L = 14.15 µm, W = 6.12 µm, Q = 1.81–2.52 (n = 150/5). **Haustoria** consisting of irregularly coiled hyphae.

FIG. 2. *Septobasidium aquilariae* (holotype, SWFC 006611). A, B. Basidiomata on branch; C. Sections of basidiomata; D. Basidiospore; E. Basidia; F. Hyphae; G. Haustoria. Scale bars: a = 5 cm; b = 2 cm; c–g = 10 µm.



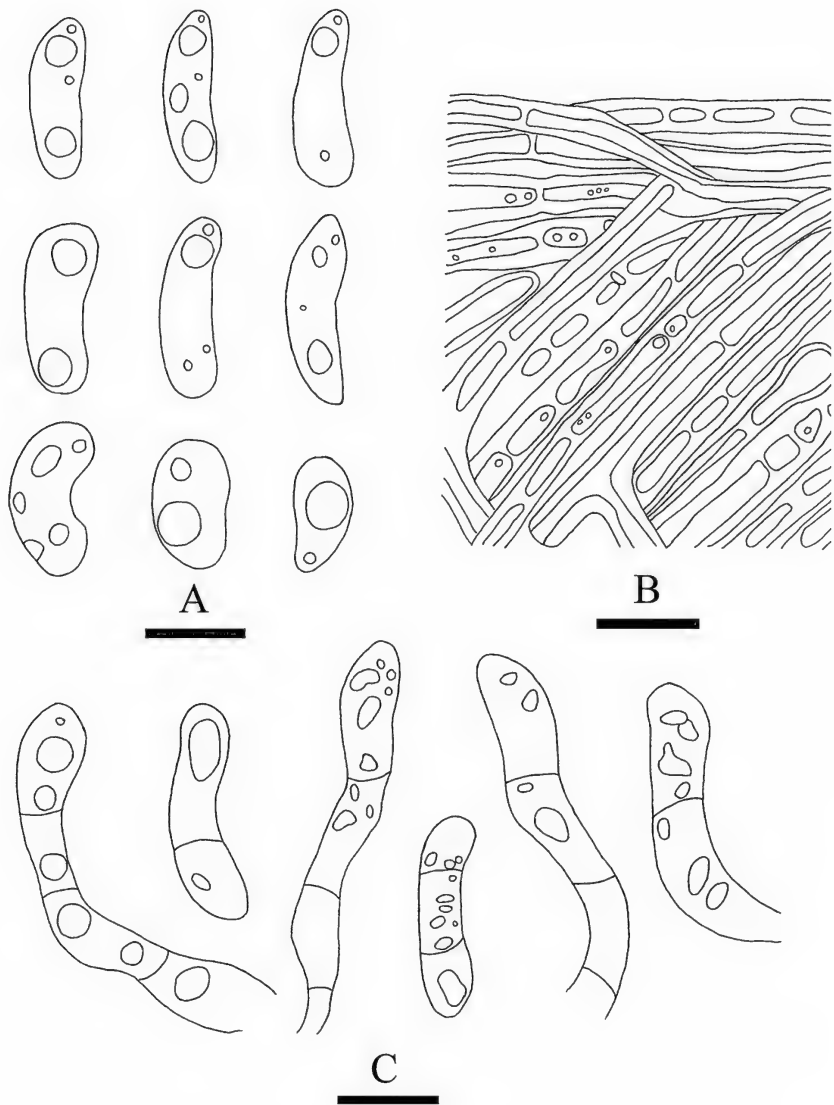


FIG. 3. *Septobasidium aquilariae* (holotype, SWFC 006611).
A. Basidiospores; B. Generative hyphae from hyphal layer; C. Basidia. Scale bars = 10 µm.

HABITAT AND DISTRIBUTION growing in association with *Pseudaulacaspis* sp. on *Aquilaria sinensis* (Lour.) Spreng. (*Thymelaeaceae*).

ADDITIONAL SPECIMENS EXAMINED: CHINA, YUNNAN PROVINCE. Xishuangbanna: Jinghong, Menghai County, in association with *Pseudaulacaspis* sp. on *Aquilaria sinensis*, 31 May 2018, CLZhao 6610 (SWFC 006610; MK802139); CLZhao 6612 (SWFC 006612; MK802141); CLZhao 6613 (SWFC 006613; MK802142); CLZhao 6614 (SWFC 006614; MK802143).

Discussion

In the present study, a new species, *Septobasidium aquilariae*, is described based on phylogenetic analyses and morphological characters.

Phylogenetically, *S. aquilariae* is closely related to “*S. cokeri*,” *S. hainanense*, and *S. maesae* (FIG. 1). But morphologically “*S. cokeri*” differs from *S. aquilariae* by its pure white hymenial surface and restricted growth on *Quercus rubra* (Gómez & Henk 2004); *S. hainanense* differs in its purple hymenial surface and larger ($25\text{--}36 \times 7\text{--}13\ \mu\text{m}$) basidia (Lu & Guo 2010a); and *S. maesae* differs by its perennial basidiocarps peeled off after maturity and larger ($28\text{--}55 \times 7.5\text{--}11.5\ \mu\text{m}$) basidia (Lu & Guo 2009a).

Several species found in China are morphologically similar to *S. aquilariae*. *Septobasidium broussonetiae* C.X. Lu & al. is distinguished by its cracking basidiocarps and growth on *Broussonetia papyrifera* (Lu & al. 2010); *S. brunneum* Wei Li bis & L. Guo differs in its purple-brown hymenial surface with many cracks and growth on *Eurya* sp. (Li & Guo 2014); *S. capparis* S.Z. Chen & L. Guo differs by its thicker ($\leq 2\ \text{mm}$ thick) section and larger ($45\text{--}56 \times 8\text{--}12\ \mu\text{m}$) basidia (Chen & Guo 2012); *S. euryae-groffii* C.X. Lu & L. Guo is distinguished by its cinnamon to chestnut brown hymenium and growth on *Eurya groffii* (Lu & Guo 2010b); *S. fissuratum* Wei Li bis & L. Guo differs in its larger ($32\text{--}45 \times 6\text{--}9\ \mu\text{m}$) basidia and growth on *Castanea* sp. (Li & Guo 2013); *S. gaoligongense* C.X. Lu & L. Guo differs in its dark brown hymenium and thinner ($260\text{--}580\ \mu\text{m}$) section (Lu & Guo 2010b).

Septobasidium guangxiense Wei Li bis & L. Guo differs from *S. aquilariae* in its yellowish brown hymenium with numerous fissures at maturity and larger ($27\text{--}38 \times 5\text{--}10\ \mu\text{m}$) basidia (Li & Guo 2014); *S. hoveniae* Wei Li bis & al. differs in its cinnamon-brown hymenium and growth on *Hovenia acerba* (Li & al. 2013); *S. polygona* C.X. Lu & L. Guo differs in its white to cinnamon-brown hymenium and growth on *Polygonum campanulatum* (Lu & Guo 2010b); *S. reevesiae* S.Z. Chen & L. Guo differs in its thicker ($1.65\text{--}2.20\ \text{mm}$) section and larger ($37\text{--}55 \times 8\text{--}13\ \mu\text{m}$) basidia and growth on *Reevesia longipetiolata* (Chen & Guo 2012); and *S. transversum* Wei Li bis & L. Guo differs in its cinnamon-brown basidiomata, its transverse

layer at the pillar bases, and larger ($42\text{--}60 \times 9\text{--}16\ \mu\text{m}$) basidia (Li & Guo 2014).

The diversity of *Septobasidium* is rich in China and 57 species have been reported, especially in subtropics and tropics (Lu & Guo 2009a,b, 2010a,b, 2011; Lu & al. 2010; Chen & Guo 2011, 2012; Li & Guo 2013, 2014). Several *Septobasidium* species have been described from Yunnan Province (Lu & Guo 2010b, 2011; Li & Guo 2013), and our new species, *S. aquilariae*, is also from Yunnan Province. It is expected that new taxa will be found after further research.

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